The Relation between Light and Pigment-Formation in Crenilabrus and Hippolyte.

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With Plate 23.

Contents.

Introductory.

This paper is a continuation of the series hitherto published in conjunction with Professor Keeble (1900–1905), and contains a further instalment of experimental results of a research upon the colour-physiology of the prawn Hippolyte varians, and the wrasse Crenilabrus melops. The work was carried out by the author during the last three years, in part at the Plymouth Laboratory of the Marine Biological Association, in part at the Millport Marine Station, and also at Manchester University. To the directors of these laboratories and to the staff of the Plymouth and Millport Stations, the special thanks of the author are due for the unstinted help which they have always been ready to give. His former colleague, Professor Hickson, has given the author ever-ready assistance and much helpful criticism.

I. The Influence of Surroundings (Algal Backgrounds) on the Colour of Crenilabrus Melops.

(1) Introductory.

The immediate object with which this experiment was undertaken was to ascertain whether in the young stage of the fish there was a sensitive period at all comparable to that which is possessed by Hippolyte. I had thought that by exposing the young fish to backgrounds of diversely coloured weeds it would be possible to obtain some light as to the origin of the colour varieties which the wrasse exhibits.

As in the case of Crustacea these colour varieties may be classed under two heads: First, the individual colour forms, which show a series of more or less marked vertical bars on a variable body colour, and second, the colour phases exhibited by any given individual.

The Labridæ offer an exceedingly rich field of research for

experiment on both these lines. It is well known that the ballan wrasse varies through a series of monochrome, barred and spotted types of coloration from deep red to blue, and it has been ascertained by Holt that a given individual is capable of passing through colour phases from a spotted to a uniform livery with accompanying changes of colour. It is also asserted (Noé and Dissard), that these colour varieties are associated with the substratum over which the fish range. Thus Gourret, in his beautifully illustrated memoir on the wrasses of Marseilles, describes the varieties of several species, associated respectively with Zostera and with Nullipore-grounds, and the seasonal changes which they undergo.

The existence of a close relation between the coloration of many animals and that of their surroundings is a well-established conclusion. In the particular and striking case of Hippolyte varians, the development of this relation has been shown by Professor Keeble and the author to take place rapidly if young transparent animals are placed with algæ in a strong light. Thus an experiment conducted in bright sunshine at Tregastel showed that out of fifteen colourless or pale red lined Hippolyte, eleven became red after two days' association with red-brown weed; and that eight out of twelve became green on green weed in the same time. Analogous but much slower changes have been established by entomologists for sensitive geometrical moths during their early larval stages. Except for this group, however, the amount of experimental evidence on the factors that determine this colour sympathy is very limited. Certain insects excepted, Crustacea are apparently the only class in which the action of the environment has been tested; and even here the light-factors that determine the development and distribution of one or more pigments so as to produce an effect in harmony with the coloration of the environment, are quite unknown.

With a view of determining these factors I undertook in 1907 a series of experiments with one of the wrasses, the common gold sinny (Crenilabrus melops).

Young specimens of ballan wrasse were unfortunately not available at Plymouth, but this species would be an even more suitable one for such an investigation.

(2) Pigments and Colour Changes.

In the case of Crenilabrus melops the coloration is of a barred type. The head is marked with streaks of colour associated with the brain and with the lateral line organs on the operculum and jaws. The tail is usually marked by a central black spot, and the greenish or yellowish trunk is traversed by six or more vertical dark brown bars which extend from the dorsal fin to the anal, but do not cover the cœlomic region. This species in its young state is the most abundant of the wrasses in Plymouth Sound.

The arrangement of the pigment is as follows: Four colouring matters contribute to this result—blue, black, yellow, and red. Contrary to the statement by Krukenberg that the blue colouring of wrasses is due to a special pigment but is an optical colour merely, I find that in Crenilabrus melops a blue substance is associated with the skeleton in such a way as to give the young animal a transparent pale blue tone when the chromatophores are contracted. The nature of this substance, which, so far as I know, has not been previously recorded, is probably not pigmental, nor has it yet been determined. The green skeleton of Belone and the "vivianite" associated with some old red sandstone fishes possibly contain allied substances. Around the blood-vessels there is also a diffused blue substance, which is most easily noticeable in the fins and the peritoneum, and forms a blue line along the aorta. The yellow and red pigments form a network derived from yellow or orange chromatophores scattered over the back and flanks and along the fin-rays. The combination of this yellow network with the underlying diffused blue pigment and the blue skeleton gives a green tinge to the young fish, whilst the expansion of red pigment gives a ruddy colouring; when both red and

yellow chromatophores are expanded a dull yellowish-brown ground colour results (Plate 23).

The vertical stripes are due to the development of black and red chromatophores along six somewhat irregular bands, beginning just in front of the dorsal fin and ending at the base of the tail fin. These bands of chromatophores are of considerable interest. They occur in their most marked form in the superb cross-striping of coral reef Labroids and other families, but they also appear under stimulation as a series of evanescent banded markings on the skin of unstriped fish. The common Crenilabrus rupestris shows this very well. When at rest it is of a nearly uniform brown or dull reddish colour, but on being handled or when transferred to contrasting surroundings the body is seen to be overspread in a wave-like manner by bars of a deeper colour, which may continue to come and go in blushes. Again, Holt has recorded the appearance and disappearance of dark transverse bars in the common ballan wrasse (Labrus maculatus). In this case the fish had exactly the same property of expanding and contracting the metameric tracts of chromatophores without altering the body colouring. Sometimes, indeed, the bands disappeared almost entirely and the fish became of a uniform green colour. Crenilabrus rupestris has, at least in its younger stages, the same property. It may, and usually does, exhibit a banded appearance, but the bands may be extinguished and the body assume an almost uniform green tinge.[1]

The presence of these bars of colour is by no means wholly dependent on the nature of the surroundings. In Crenilabrus melops they tend to appear under conditions that favour expansion of pigments, but they also appear instantly if a fish is transferred from white to dark vessels. Tactile stimuli are especially effective in bringing about alternate flushing and pallor along these tracts. It is clear that they are more or less metamerically arranged tracts along which

[1] Since this was written the observations of Townsend (1909) and of Tate Regan (1909) have revealed an unexpectedly wide range of rapid colour-changes in tropical fishes.

nerve impulses act on the chromatophores. The phenomena, in fact, recall the pilo-motor or goose-skin reflex in man. Recent physiological researches (Van Rynberk) have shown in certain Pleuronectids that the ganglia of the sympathetic system supply each a definite transverse band-like region on the upper side, and that these regions overlap one another to the half of their width. Stimulation of these regions by induction currents produces contraction of the chromatophores. Section of the spinal nerves and of the rami communicantes of the sympathetic, leave the regions in question dark and their chromatophores permanently expanded.

This power of localised colour change is still very imperfectly understood. The development of the affected regions has not been undertaken, nor is the heightened coloration of the breeding season as yet in any way explained. It is therefore of some interest to note that in the case of the uniformly coloured adult Ctenolabrus rupestris I have been able to observe the banded pattern appearing in the post-larval stage 10 mm. long. The pattern at that stage differs but slightly from the livery of "melops," but the difference is that in the former the pigmentation arises in the form of these transverse bars separated by clear areas, whereas in melops, so far as I have observed the species, the barred pattern is inter-connected by diffused chromatophores. The subsequent monochrome pattern of "rupestris" is evidently derived from this earlier-barred one by development of interstitial pigment: but the presence of the bars even in the adult is revealed at the moment when under stimulation the skin becomes traversed by dark segmental bars alternating with areas of pallor.

(3) The Influence of Daylight reflected from the Algal Backgrounds.

Experiments on this problem were carried out as follows: The young wrasses, obtained by hand-netting over Laminaria fringes, were placed in clear glass vessels, and these, in turn,

were immersed in bell-jars filled respectively with Laminaria saccharina, Nitophyllum, and Ulva. Similar batches of wrasses were also placed in dark-bottomed and porcelain vessels, and in complete darkness. A double circulation was maintained, and the weeds were renewed twice a week. The young fish were fed with tow-nettings and with amphipods. The bell-jars stood on the south side of the laboratory, and received diffuse daylight on all sides.

Table I, pp. 574–575, gives the result of this experiment, which lasted for about three weeks.

The light reflected from the weed backgrounds is a most important factor in the case, and in previous experiments has not received sufficient attention. Different as the three weeds are to the naked eye, their spectroscopic examination reveals little diversity; indeed, the important differences in the light reflected from their surfaces (or transmitted through them) is the preponderance of one or more of the parts of the spectrum they transmit in common. Thus the green Ulva (in more than one layer) transmits from red to green, the green being somewhat more vivid than the red, but with no great difference of intensity. The red Nitophyllum also reflects red to green, but whereas the red is bright the green is exceedingly dim. Laminaria also transmits from red to green, but here the whole spectrum is very faint.

The results show that brown weed backgrounds produce the same effect on the coloration of young Crenilabrus melops as does a black background. The fish may undergo temporary flushing and pallor under the conditions of examination, and there is a tendency for the dark bands to lose their distinctness, but the result (Pl. 23, fig. 1) is decisive. The amount of red pigment is greater than in similar specimens exposed to light reflected from red or green weed. The reflected light is more dim and is diffused over the whole spectrum in the case of black backgrounds than in that of the brown weed, and it is probably this difference which explains a tendency to greenness in some of the records.

The results with green and red weeds en masse are some-

what surprising. The fish in both cases become green or greenish, with brown bands. There is no well-marked differential result, such as we shall find in dealing with transmitted light. The yellow pigment is well developed and well expanded; the red pigment, however, showed more expansion in green backgrounds than in red. This coloration is one intermediate between a white and a black background result. In the case of red weed the effective rays are the red or the red-orange, and so far from these encouraging the develop ent and expansion of the red pigment they seem to have the contrary effect, for from August 14th to 20th the records all run green, and though there is a subsequent period of darkening the red colour is not noticeable. The inference, therefore, is that in the case of red weed the red end of the spectrum is concerned in the formation and expansion of yellow pigment. In the case of green weed the results are so similar as to leave the specific action of the green rays uncertain. The red and orange rays, both of green and of red weed, appear to act alike, while the bright green rays of Ulva or the dull green of Nitophyllum does not exert any very definite action.

(4) Influence of Light transmitted through Algæ. (Table II, p. 576, and Pl. 23, figs. 2 and 3.)

When, however, the experiment of transmitting light through a thin layer of algal tissue is made, the results are not only more definite but also help to interpret the former experiment.

Table II gives the records obtained by exposing young wrasses of the same species as those employed for the preceding work, to light transmitted through two fronds of green, brown, and red weed respectively.

For this purpose two rectangular museum jars were employed. The inner one contained the fish, and was separated on three sides by a chink about 1 cm. wide from the outer, the space being filled with water and fronds of the

weed. The whole stood in strong diffused light and had a double circulation.

These experiments give a much more definite result. In green weed surroundings three of the fish lost in a week their initial greenness, and, together with the remainder, became entirely brown. Not only so; out of four, three showed considerable amounts of red pigment, and, as we shall see, contrasted very markedly with the other experimental batches (fig. 3).

The brown weed experiments gave a curious result. The fish were initially green and so remained, but in the red weed, out of five specimens, two of which had been originally greenish, three were now green and the remaining two showed only tinges of brown, although at first they had been barred with that colour.

In this experiment, therefore, green and red weed acted quite differently from each other; the green light-filter encouraged the brown colour and red pigment, whereas red encouraged green colour and yellow pigment. Brown surroundings resembled red ones in maintaining the green tint. The contrast between this result with transmitted light and the former with reflected light is so striking and puzzling that I, at this point, undertook the experiments (referred to on pp. 552–561) on Hippolyte with a view to clearing up the discrepancy.

The explanation, I believe, is to be sought in the spectroscopic analysis of the light transmitted or reflected by thin and by thick masses of the respective weeds. Thus Ulva, two fronds in thickness, transmits red to green, but as the thickness is increased it transmits orange, yellow, and green only. Single fronds of Nitophyllum transmit red to green, but several transmit almost pure red with a trace of green. Brown weeds transmit from red to green, the general intensity being low. Taking, therefore, the red as the purest screen, the remarkable feature about it is that along with the greater purity and intensity of the red light there is in the fish submitted to its action a green result due to the combina-

tion of many and well-expanded yellow pigmented chromatophores with an underlying blue pigment. The light, it is true, is not monochromatic, but in the succeeding section it will be seen that a similar result obtains even when monochromatic light is used.

Here, then, the result is that in strong red-orange light yellow pigment is well developed, but that red pigment is not. Turning to this green-weed experiment we have the converse conditions and result. In these fish a brown colour and red pigment are strongly developed (Table II).

Considering that the contrast of green weed to red weed lies in the extension and greater brightness of the green part of the spectrum, the inference is that the development of red pigment is due to the green light and that the strong red light encourages the formation of yellow. The two together give a brown coloration.

Brown weed in a thin film transmits from red to light blue, but only the red end is of fair intensity. Under a brown screen the fish maintain their green colour and the contracted condition of the red chromatophores.

(5) Note on Coloration of Larval Gobies.

Before passing on to these experiments on Hippolyte, I may interpolate a short statement of results obtained by subjecting certain larval fish to varying illumination. It is to be expected that the discovery of sensitive and responsive species will prove a difficult matter, and these notes may serve to help future workers in their choice of material.

During the past summer I obtained the eggs of two species of Gobius (G. paganellus and G. minutus) with a view to determining the rate and direction of pigmentary response to cultural conditions. The larval chromatophores are either black or of that "yellow" colour which is only seen by reflected light. Specimens developed and hatched in darkness showed normal pigmentation. On green backgrounds (obtained as explained on pp. 561, 562) the appearance of the larvæ after a week's exposure and again after a fortnight

was not perceptibly different from that of those kept on red backgrounds, nor from control specimens kept in clear vessels uniformly illuminated. Larvæ of Lepidogaster gonanii and of L. bimaculatus were equally intractable. There can be no doubt that long-continued experiments are necessary owing to the slow pigmentary changes in these animals.

(6) Summary.

(Crenilabrus melops.)

Darkness (for five days) produces extreme contraction of all chromatophores.

Black and white backgrounds in white light (three weeks' exposure) gave respectively the usual dark brown and the light (green) colouring associated with this illumination.

Brown-weed background acts like black. Green and red algal backgrounds produce a greenish tint intermediate between the effects of black and white backgrounds.

Weed light-filters produce an entirely different effect from the weed backgrounds (Table II). Daylight transmitted through green weed induced brown coloration and considerable amount of new red pigment. Daylight transmitted through red weed produced green coloration and yellow pigment. Brown weed is too opaque for any differential effect to show itself.

These results, then, go to show that the action of algal backgrounds is complicated by the impurity of the colours transmitted or reflected from them. The nearer these approach to the complexity of white light the more does the background resemble a black or a white one, i. e. general contraction or expansion results. The purer the colour or the more intense the particular part of the spectrum, the greater is the development of pigment of complementary colour. These results, however, were too few to establish the relation, and I therefore undertook the following experiments on a more convenient subject, Hippolyte varians.

II. The Colour-physiology of Hippolyte Varians.

Previous analysis (1904) of the factors that determine the wide range of sympathetic coloration in Hippolyte varians has revealed :

(1) That at the time of birth the chromatophore system is constant in organisation, and offers such slight variations in the amount of the only true pigment (red) as to suggest that the colour of the parent does not influence that of the offspring.

(2) That the next known (adolescent) stage (4·5 mm. long) presents three colour patterns : Red-lined pattern (by far the most common), barred pattern (rare), and monochrome pattern.

(3) That these young, transparent, adolescent animals become green on green weed, or red on red weed within forty-eight hours if placed amongst a mass of weed strongly illuminated by direct sunlight.

(4) That having assumed the tint of their surroundings the young animals can be persuaded to change it without difficulty, but that in later stages this elasticity is lost and colour-change is only effected slowly or not at all.

(5) That the adolescent colour-pattern may become the adult one if the environment is kept constant, but that lined and barred patterns are in all probability transformed into a monochrome by the filling up of the interstices upon exposure to a more uniformly coloured background. These results place the chief efficacy of colour-development in Hippolyte upon external factors. The eye and nervous system control the response to background, but do not determine it. Inheritance provides paths along which pigment develops, but does not settle the colour or pattern. The young animal appears plastic, but the old one is a creature of habit.

The need for a more careful analysis of these responsible external factors and of their continued working has led to the following results. The variability of broods born of similarly and of diversely coloured parents and the prolonged action of

monochromatic light are the two chief problems dealt with here. The conclusion is drawn that when monochromatic light is made to fall upon all sides of the experimental animals, so as to obviate a strong background effect, the result is a pigmentation complementary to the colour of the incident light and also to that obtained in Hippolyte by the use of coloured backgrounds and white light.

(1) Methods.

Although none of the methods employed for rearing the larvæ of Hippolyte were thoroughly successful, the record of the attempts made on this very difficult problem may be of assistance to other workers. A means of obtaining a satisfactory solution is one of the most pressing needs of experimental biology.

The vessels used consisted of large bell-jars, supplied with an air- or water-current or stirred by a glass plunger. Seasoned vessels as well as sterilised ones were used; filtered, "outside," and tank-water were respectively employed; diatoms (Nitschia) and algal cultures (Phæocystis mixed with other green flagellates) were used as food. The vessels were shaded, exposed to diffuse light, and kept in darkness; the backgrounds were translucent, absorbent, and reflecting; the incident light used was monochromatic (red and green) as well as white light. The temperature was kept down to 16° C. by a water-jacket, and in other cases allowed to rise to 18° C. or over, but in spite of all these variations of treatment the larvæ only survived about ten days. It is possible that some means of removing the first sickly specimens would be a great improvement, and it is, of course, also likely that a better diet could be found. The larvæ, however, readily eat green flagellates and seemed to digest them.

The monochromatic screens used in the case of larvæ consisted of selected pieces of coloured glass (ruby or green) combined with coloured gelatine films. These were placed over the inverted bell-jars, the sides of which were converted into absorbing or reflecting backgrounds.

A continuous air-current was led into the water, and the coloured screen was cut so that its halves embraced the air-tube, which was blackened at this point. The junctions of the screen with the bell-jars consisted of black velveteen so as to cut out any oblique white rays, but it was found that great care is needed to avoid liquefaction of the gelatine films. A trial was made with Schott's coloured glass, but except the red the samples submitted were not monochromatic.

In order to observe the prolonged effect of monochromatic light, and to obviate the dominant influence of the background, fluid screens were constructed. To insure a fairly strong light the screen was made of one cell only, and not, as in the case of Landolt's original design, of two or more. A double glass vessel was employed consisting of two beakers or of two large cuvettes, the inner one standing on glass supports, so that its rim just cleared that of the outer vessel. The inner vessel was then provided with young, transparent Hippolyte in filtered water, and finely divided Ceramium was used as food. The space between the two was then filled with the colour filter until the level exceeded that of the water in the inner vessel, the top inch or so of which was rendered opaque. A cover of glass, or of glass and gelatine, was then placed over the double vessel, and the whole was then transferred to a shallow aquarium in a strong light. In one case a circulation of tank-water was maintained in the inner vessel. The main point of the apparatus is to provide a means of flooding the animals (which remain in mid-water attached to their weed) with transmitted coloured light, and thus largely to avoid the affect of light reflected from an absorbent or reflecting background, such as has been generally employed in previous experiments. The surfaces on which the vessels stood were either slate or dull white brick, but there was always a layer of the fluid, some 2 cm. in thickness, btween the bottoms as well as between the sides of the two vessels.

The coloured solutions employed consisted of the following: For red a strong solution of erythrosin in distilled water, the strength being increased until a 2 cm. layer cuts out all the

orange. Weak lithium carmine solution in a 2 mm. layer was used in 1909. For green a 60 per cent. solution of copper chloride with a trace ($\frac{1}{20}$ of the volume employed) of 6 per cent. potassium chromate gave a good result in 1·5 and 2 cm. thickness. For blue, ammoniacal solution of copper sulphate was used, a concentrated solution to which strong ammonia was added until the precipitate was thrown down and could be filtered off. Unfortunately this blue screen, probably owing to the ammonia exhaled, is very toxic.

The light employed was direct, or direct and diffuse, daylight. In the former case the vessels stood for more than half their depth in a tank placed on the south side of the Plymouth Laboratory. In the latter the vessels were placed about 10 ft. from the south window on the slate base of the table tanks. In 1909 the vessels stood opposite a north window on a glass shelf, and were illuminated from below by a mirror as well as from above and laterally. The temperature maintained by a flow of water around the outer vessel was 16·5° to 17·5° C. even in direct light; that of the inner vessel with a continuous water-current was 16° to 17° C. Other experimental batches were maintained in clear glass, and under white or black background influence as well as in darkness.

(2) Variability of the Larval Pigment.

The chromatophores of Hippolyte varians at the time of hatching usually contain a single granular pigment of a red (scarlet) colour. No true yellow pigment is present, but there is a substance in the chromatophores that is yellow by reflected light and brownish by transmitted light. This is very constant in all broods. A variable amount of diffuse blue pigment is associated with the red.

Previous investigations (Gamble and Keeble) have shown that " the progeny of females (in Hippolyte varians) with much red pigment have more of this substance in each chromatophore than have those derived from green parents in which red pigment is less abundant.

The question is of some importance since the initial amount

of this substance might conceivably influence the subsequent colour-history. It seemed, therefore, advisable to obtain more records of this varying proportion of red pigment, and also to determine the conditions which favour or inhibit its development.

The results obtained are shown in the adjoining Table, and are derived from a study of the offspring of some twenty parents. Larvæ from those Hippolyte which are pink, red, or brown, possess a fair amount of the red pigment in their chromatophores at the time of hatching (the greatest amount in the samples examined being in the red-lined female broods). Larvæ from colourless (extremely pale pink) female varians are devoid of red pigment; whilst larvæ of green parents occupy an intermediate position, some batches being coloured like those of brown or pink parents, but not so deeply; others from equally good green parents exhibit no red; and others, again, exhibit, unlike all the preceding cases, an inconstancy, and show traces of the red pigment in only 36 per cent. or so of the offspring.

Hippolyte varians.

Number and colour of female parent.	Amount of red pigment in the just-hatched larvæ.
2. Red-lined forms . . .	Much; constant.
3. Pink	Fair amount: constant.
1. Pink	Traces only in some, fair in rest.
3. Brown	Fair amount: constant.
6. Green	Fair amount; constant.
2. Green	Traces in 36 % of larvæ examined.
2. Green	Absent.
1. Almost colourless (pale pink) .	Absent.

These results are of interest in several ways. They confirm on the whole the earlier conclusion that excessively red colour in the parent is associated generally with excess in the early larvæ; but they also show that the offspring of green female varians show two types of coloration, namely, that with some red pigment and that with none; and further that the two

types may be combined in a single brood in the proportion of 36 per cent. dominant or pigmented forms.

This result at once suggests that green in the parent is of twofold origin; and the facts of earlier experiments support the suggestion. It has been shown (Gamble and Keeble) that green is both an independent stable colour-form and also a colour assumed by brown specimens on a transfer to green weed. Further experiments are necessary to decide whether the green parents with recessive red colouring are of the former type, and those with more dominant red pigment are of the latter colour-history. The new points that emerge are the absence of red pigment in certain broods, and its presence in only a percentage of others.

Repeated attempts were made to experiment with broods from an isolated parent under diverse conditions of light, food, and temperature, but without much success after the first week or ten days. The chief results obtained were (1) that zœæ developed and hatched in darkness (from brown parents that became green under these conditions) possess the normal pigmentation, thus showing that light is not essential to pigment development, and also confirming the suggestion just made, that it is those green parents which had been previously brown that give rise to larvæ with red pigment; and (2) that there is a steady increase in the amount of red pigment in broods of green parents. For example, the tint of zœæ of green parents approximated after a few days to the colouring of the larvæ of red parents. It is, therefore, doubtful whether the initial differences in pigmentation between the broods of similarly or diversely coloured parents are of any moment in determining the ultimate coloration.

III. (1) The Influence of Transmitted Monochromatic Light on the Formation of Pigments in Hippolyte Varians.

Previous work on the influence of monochromatic light (1900, p. 619, 1904, p. 356) upon Crustacea concerned itself

chiefly with short exposures made upon an absorbing or reflecting background. The results showed that the light acted irrespective of its colour according to the nature of the background, almost as though it were white light of low intensity. Moreover, experiments with coloured backgrounds of weed, against which young, transparent, almost colourless Hippolyte were exposed to direct sunlight, showed (1905, see Tables) that in two days, eleven out of fifteen prawns became red on red weed, and eight out of twelve became green on green weed. The coloured backgrounds, when flooded with white light, produced sympathetic colouring. The red was a mixture of red and yellow, the former predominating, the green a mixture of the same two pigments but yellow predominating. In both cases a diffuse blue pigment occurs also.

This result appeared to lend some support to the view of Wiener (1895) (which has since undergone elaboration [Bachmetjew, 1903]), and to suggest that the dominant rays of the background evoked especially that pigment or that group which agreed in colour with the reflected light.

In order to ascertain more fully the effect of monochromatic light, I determined to eliminate, as far as possible, this dominant influence of background, and to ascertain the result of exposure to incident light of one colour. So far as I am aware, the experiment in this form has not hitherto been undertaken. The starting-point for this experiment was furnished by young transparent Hippolyte varians taken by netting over Zostera beds and Laminaria-fringes. These fall into two groups: typical faintly red-lined forms provided with red and yellow chromatophores along the gut and nerve-cord, and with red ones at segmental intervals in the integument; and more uniformly coloured specimens with similar pigments, but with chromatophores more evenly distributed. In both cases the amount of pigment is not enough to give the specimens a decided tinge. They are similar to those used for the weed background experiments quoted above, and are figured on Pl. 23, fig. 4.

The vessels which were used are described above (p. 554),

and the conditions of the experiment were such as to flood the animals with monochromatic light on all sides. The weed chiefly used for food was the natural food-plant, *Ceramium*; a little fine green weed was used in one of the red light experiments. The vessel was surrounded on three sides by the fluid colour screen and rested on a faintly reflecting surface, so there was no strong background effect. The light employed was direct and diffused sunlight, and the effects of heat and of ultra-violet rays were largely obviated by the conditions of the experiment.

The results of the experiment are given in Table III, pp. 577, 578, and show that whilst the *Hippolyte*, in white light, developed into brown forms containing both red and yellow pigment in about equal proportions, those in red light passed through a brown stage, but ultimately (three weeks) became green, some remaining, however, reddish-yellow in 1909, whilst the survivors in green light became bright carmine. In other words the ultimate colour in this experiment is the complement of that of the incident light.

The details of the end-result show clearly that the green *Hippolyte* produced in red light and the crimson *Hippolyte* produced in green light are peculiar and distinctive. The former possess yellow pigment in a maximally expanded state, and such little red as they possess is of a vermilion tint. Moreover, the yellow is of a distinctly greenish tinge and is accompanied by either very little diffuse blue or none. Thus the green colour in these experimental specimens under red light is largely due to an increase in the amount and quality of the yellow pigment accompanied by contraction of the formerly dominant red pigment (Pl. 23, figs. 6 and 9).

The crimson *Hippolyte* produced in green light is no less distinctive (Pl. 23, fig. 5). In contrast to the usual type of red forms, the yellow pigment has completely disappeared and the chromatophores are entirely filled with a deep carmine pigment suffused with a bluish tinge. The general deep carmine colour was new to me. Moreover, the chromatophores on the surface of the eye-stalks were abnormally developed.

In view of this very decided complementary colour-change the regrettable mortality that occurred in vessels exposed to green light in 1908 does not seriously diminish the value of this result, though larger numbers would add to its cogency. These were obtained in 1909. The experiments of 1908 and 1909 are compared on Table V and with the other experiments of this paper on Table IV.

(2) The Influence of White Backgrounds and of Monochromatic Backgrounds in White Light.

The effect of short exposures to the influence of white (porcelain) and of black (cloth or paint on glass) backgrounds on the colouring of young and old Hippolyte has been fully treated in a previous memoir.[1] It was there shown that whatever the quality or quantity of the light employed (within the experimental limits), the background effect dominated, producing contraction if white and expansion if black. It occurred to me, however, to see whether the same results would follow a long exposure made with young specimens in which the pigments were rapidly developing.

The results of a month's trial are of considerable interest. The Hippolyte on black surfaces simply followed the usual procedure under such conditions, and developed maximal amounts of red and yellow pigments, which gave them a deep reddish tint. On the white background, however, after a first phase of transparency, they began to develop red pigment along the nerve-cord, and finally became uniformly marked with a ventral red stripe, whilst over the rest of the body the pigments were reduced to microscopic dots or disappeared. This remarkably adaptive result was obtained in diffuse light, the top of the deep porcelain vessel being covered with muslin, through which a stream of water was maintained from a tank above (Table IV, p. 579).

In 1909 these background effects were extended so as to include the results of red and green. The vessels employed were large museum jars, painted, except for a large rectangular

[1] (1904), p. 353.

window, with several coats of pure paint. Spectroscopic tests showed that the red was pure and that the green paint reflected only a trace of blue in addition to the whole of the green light. These vessels were kept under a water-circulation and faced a south light. Finely divided pieces of Ceramium were employed as food. The Hippolyte used were small, almost colourless specimens, similar to those employed in the other experiments on coloured light.

The results of exposure to these monochromatic backgrounds was very decisive (Table IV, p. 579). Upon the green one the development of pigment was arrested. The Hippolyte assumed a semi-nocturnal (green) tint, and remained with the red pigment contracted throughout the experiment. This green colour is, however, not retained if the background is changed. Under these circumstances the animals revert to the pale red-lined colour variety which they exhibited initially. Upon the red background, on the other hand, the red and yellow pigments had considerably developed, and after a month's exposure gave a bright orange-red tint to the specimens, and this persisted after change to other backgrounds. It would be of interest to know whether Minckiewicz (1907–8), who has also obtained results of this kind with Hippolyte, tested the permanent or transient nature of the induced colouring.

IV. The Food of Hippolyte as a Possible Source of Pigment.

The relation of Hippolyte varians to the algæ of its choice is a distinctive one. The peculiar features of this species, the range and cryptic character of its variable coloration, its choice of, and tenacity of hold upon its weed, its distribution, and its food are all bound up with the presence of these plants. It is possible that Idothea and some Amphipods are equally intimately related to their habitat, but among macrurous Decapods Hippolyte varians is probably unique in this dependence upon its algal environment.

In former papers on the subject, the relation existing between the pigments of Hippolyte and the coloration of its surroundings was explained as due to light effects, as if the weed backgrounds in virtue of their disposition, of their luminous character, and colour, acted as stimuli to the chromatophores of the prawn. However, before we accept that explanation, the influence of two other factors must be considered: First, the effect of darkness on pigment-formation, and second, the source of these pigments, whether derivative or not. The first factor—darkness—is discussed on pp. 577–579, and it is there shown that the red (vermilion) pigment does not require the stimulus of light for its development, and that it increases in amount when the Hippolyte are kept in darkness. The yellow pigment, however, is more dependent on light for its formation and increase, diminishing in amount in specimens kept in darkness, especially if little or no food is supplied to them. The crimson pigment and the diffuse blue colouring matter are not at present investigated from this point of view. There is evidence, however, that light is essential to the production of all varieties of Hippolyte, except the reddish-brown ones. The other factor—the source of pigment itself—is less known than are the conditions which determine each particular tint. The colouring matter of the food is one possible source, and this has to be briefly considered, since, if proved, it would simplify the problem of sympathetic coloration. That the sub-hypodermal colours of caterpillars and beetle larvæ are due to diffusion of fatty pigments from the food-contents of the gut is a conclusion reached both by Poulton and Towers, though the physiological details of this remarkable process have never been ascertained. But the hypodermal colours of these animals are of an entirely different nature from those of Hippolyte, and appear to be determined by enzymes, elaborated by this layer acting upon the "primary" cuticle or retained within the hypodermal cells. In Hippolyte and in Crustacea generally (as in the insect larvæ), the first formed pigments are developed independently of the plant-food present in the

mother, and it would be of great interest to know how they were formed.

In order to test the influence of food-pigments on the development of pigment in Hippolyte, the following experiment was carried out at Millport, N.B. A series of double glass vessels were prepared, the Hippolyte being placed in the inner chamber and a mass of weed in the outer one. Two series of pressure-bottles, one in diffused light, the other in darkness, were set up for isolated specimens. The food employed was chosen from the following: The natural alga chopped up into fine pieces so as not to act as a massive background; etiolated Laminaria, also subdivided; the muscle of Hyas, the colourless ovary of Hyas, and the scarlet, mature ovary of the same crab. The specimens of Hippolyte employed were 5–7 mm. in length, colourless, and tending on a black background to assume a faint brown-lined colour pattern.

TABLE A.—Feeding Experiment.

Colour of Hippolyte after exposure to contrasted colours in food and surroundings, Millport, 1909. Colourless foods employed are crabs' muscle, etiolated Laminaria, and colourless ovary of Hyas. The Hippolyte used were from 6–8 mm. long and colourless to the naked eye. Experiment lasted seven to ten days.

Colour and nature of surroundings.	Colours of foods employed.			
	Colourless.	Red (scarlet ovary).	Brown (algæ).	Green (algæ).
Darkness	Pale brown-lined	Reddish-brown	—	Reddish or colourless, 1 green.
Green algæ	Green	Green	2 green, 1 brown	—
Red algæ	Pinkish Greyish	—	—	—
Brown algæ	2 brown, 1 grey	—	—	—
Parti-coloured yellowish oilcloth	Black-lined	Brown-lined	Brown-lined	Brown-lined.

The results are shown on Table A, and at once bring out the fact that colourless muscle, white or red, ova are greedily taken up, but that the background is the dominating factor in the resultant coloration in daylight. Thus against a background of green weed Hippolyte fed with colourless food, with red ovary, and with fine brown weed became green. In darkness, however, the amount of pigment in the food has a rough relation to the resulting colouring that will need further experimental testing, but there is no good evidence that the colour of the food determines that of the prawn.

V. Analysis of the Coloured Light Experiments.

(1) In green light, and amongst red weed, Hippolyte develops crimson and deep, not superficial, colouring.

The presence in the experimental vessels of a fair quantity of finely branched red weed (Ceramium) would, under the action of diffused, strong green light act as a black background, and this, as we know, in the presence of white light, encourages the formation of vermilion and yellow pigments, and these are most notably absent.

The crimson effect in green light cannot, therefore, be merely due to dim light acting on a dark background. It must be due to a distinctive factor not present in the other experiments, and that factor can only be the green rays. In the presence of these rays, not only is the crimson pigment developed, but the vermilion and yellow pigments are dismissed. Whether a similar result would follow if a colourless food were used is of course a subject for further research.

The most striking feature of this crimson colouring obtained during exposure to green light, is the fact that it is complementary in colour to that of the incident light. This relation may have a considerable significance. In an earlier paper (1905) it was pointed out that strongly insolated Hippolyte showed mobile fat in their chromatophores, and as this fat disappeared in specimens transferred to darkness there was some ground for the inference that the production of this

fat was associated with the presence of light. If that were so the assumption of a complementary colouring would be obviously the best means of absorbing the maximum amount of coloured light, and of obtaining any other benefit which light might confer upon metabolism. Under the conditions of deep water, where the green or green-blue rays have filtered down from the surface, such a colouring would be the most efficient absorbing pigmentation, and it is well known that in hauls made from the deeper water of the English Channel the *Hippolyte* are uniformly of a crimson colour.

The facts as to these crimson *Hippolyte* produced in green light would be most comprehensively explained by saying that the red *Ceramium* acted merely as an excitement to coloration, but that the carmine pigmentation is produced under the direct stimulus of the green light employed.

(2) Red light	Green weed .	Green coloration .	Superficial and deep.
	Red weed .	Yellow or brownish-yellow	chromatophores.

The action of red light is less easily analysed. The constant effect associated with it, is the production of yellow pigment and the maximal expansion of that pigment into networks producing a grand colour. Then, according to the absorbent or reflecting nature of the background (i.e. green weed or red weed), we have a green or a brownish tint, in the latter case accompanied by a development of scarlet chromatophores both at the surface and along the lines of the alimentary tract and of the nerve-cord.

In the case of red light, therefore, it would seem that the direct action of the rays lies in the production of yellow pigment, and that the nature of the background, indirectly modified by the further action of the red light, modifies this yellow coloration less or more. If the background be red, the action of the rays is intensified, and a red background is thus instituted. Probably this is the factor that gives the scarlet chromatophores, for, as will be seen subsequently, that is the effect of a red background in white light; the resultant colour is then brownish-yellow; but where, as in

the case of green weed, the background is of a less luminous character, the red colour contracts in the Hippolyte and the resultant coloration is then green, owing, in some cases, to the presence of diffuse blue mingling with the yellow network, and in the longest experiment to an apparent change in the pigment from yellow to green. The most important and most clear influence of red light, however, is the spread of the yellow pigment.

These results are so strikingly dissonant from those obtained by subjecting Hippolyte to green or to red backgrounds that an explanation is clearly called for. They differ not only in being totally opposed to the sympathetic colouring so characteristic of the latter, but also in being slowly acquired. It may fairly be asked, if red light reflected from red surroundings gives red Hippolyte, why does red light diffused give green or yellow ones? The same contradictory relation obtains between the action of green surroundings and diffused green light.

In answer to this objection attention may be drawn to the double nature of the light affecting Hippolyte under natural conditions. There is the light reflected from the background and there is also the general diffuse light.

The rapid sympathetic background colour-relations obtained experimentally have been made in strong daylight, and as the depth of water is increased or as the red end of the spectrum is cut off the conditions of the experiment are materially altered. A strongly coloured background becomes black in every light except that of its own colour, and in the presence of it we should expect the usual black background effect (brown, i.e. red and yellow pigments) to be produced in Hippolyte in any light except that with which it agreed in colour. But whilst this background effect is an undoubted factor, its potency is determined by another factor, namely, the definite action of diffused monochromatic light. The action of many rays has yet to be determined, but from the foregoing account a case has been made out for the action of green and of red light. This action, though slow, is very

precise, and it would certainly help to account for the crimson and yellow colouring found in deep-water and shallow-water Hippolyte respectively.

The results, then, of these two factors, the action of diffused coloured light and that of backgrounds in white or monochromatic light, are not contradictory. They are the two factors which, so far as we yet know, are associated in the production of pigmentation in Hippolyte. The green specimens on Zostera are green, not only because they are on a green background in bright or fairly bright light, but because at or near the surface of the sea the red rays are most potent, and their action is to produce that network of yellow pigment in Hippolyte, which is the basis not only of green tints but of those yellowish tints that this animal assumes on the etiolated parts of Zostera, and of the brown specimens on various brown weeds so characteristic of the Plymouth littoral flora. The diffuse red light, on penetrating to more densely absorbing backgrounds, such as coarser brown weeds, is checked in its action upon Hippolyte by the tendency for such backgrounds to produce red pigment in them. Hence the absence in such cases of that more precise colour-relation to the incident light. The brown pigmentation contains many red and yellow chromatophores, but the red is scarlet and not the crimson of the deeper zones.

Passing out of the range of the action of the red rays, the characteristic zone of the Florideæ is encountered, and it is in this zone that the green rays are more potent. Their effect in producing crimson pigmentation is seen in parti-coloured specimens of the red-lined variety and in occasional pink specimens of the Laminarian zone, but it is not until a fair depth is encountered that their action is made clear by the dominance of this peculiar carmine pigment, which has hitherto been confused with the vermilion or scarlet one under the confusing term "red." No doubt there are similar effects of yellow, orange, and blue rays to be analysed before a full analysis of the coloration of Hippolyte can be given. The main conclusion derived from these experiments is that

Influence of Light on the Colours of Lepidopterous Pupæ. (After Poulton, Petersen, etc.)[1]

Light.	Colour of background.	Spectrum of background.	Resulting colouring.		
			Vanessa Io.	Pieris rapæ.	P. brassicæ.
None	—	—	Irregular (dark and light)	—	Irregular.
White	Red	Red	Darkest)	Dark (Poulton), Light (Peterson)	Dark green.
—	Orange	Red to yellow	Light (green)	Green	Green.
—	Yellow	Red to green	Very light (green)	—	—
—	Light green	Red to green (red to yellow largely absorbed in some experiments)	Light (green)	Light (green)	—
—	Dark green	General absorption least in green	Dark (brown)	—	Dark.
—	Blue	General absorption least in blue	Dark	—	—
Red (pure)	White	—	Light (green)	—	Green.
—	Light wood	—	Ditto	—	—
—	Orange	—	—	—	Intermediate.
Red (red and some yellow)	White	—	Light (green)	Green	—
—	Light wood	—	—	—	—
—	Dark	—	Unknown	—	—
Yellow (red to green)	Light	—	Green	—	Light.
—	Dark	—	—	—	Darker.
Green (pure), green glass	Plain wood	—	Green	—	—
	White	—	Light (green)	—	—
	Dark	—	—	Dark	—
	Green	—	Darkish (V. urticæ)	—	—
Green (some red, yellow, and green)	White	—	Darkish	—	—
	Red	—	Ditto	—	—
	Orange	—	2 light, 3 dark	—	—
	Blue	—	Dark	—	—
Blue. (General absorption least in blue; some red, yellow green and blue rays are transmitted)	Light	—	Ditto	—	Dark.
	Dark	—	Ditto	—	Ditto.

[1] The references to these papers are given fully in Bachmetjew's work quoted on p. 582. See especially 'Trans. Entomol. Soc. London,' 1892.

the pigments developed in Hippolyte, when kept in diffused monochromatic light, are not the same as those which appear in specimens kept in daylight on a background reflecting these rays. On a red background in white light, Hippolyte becomes reddish-orange; in pure red light it becomes yellowish or green. On a green background in white light Hippolyte becomes pale green. In pure green light it becomes crimson. On backgrounds of weeds, young colourless specimens speedily acquire the corresponding tint. Monochromatic light, then, when saturated, has an entirely different effect from the same light diluted with daylight. As we pass from the surface to the deeper waters of the sea this dilution becomes less marked. The "background effect," so potent in producing the more littoral colour varieties, becomes less overwhelming as the red and yellow rays are absorbed by the surface waters. Further down, in British coastal waters, the blue end of the spectrum is said to be absorbed, so that at eight fathoms the dominant light rays are greenish or bluish-green (Oltmanns [1]). Consequently the effect of saturated monochromatic light is most probably felt in the region below the eight-fathom line.

If this distinction between the effects of coloured backgrounds in white light and of diffused monochromatic light on pigment production is well founded, it should be supported by analogous results in other animals. Fortunately the work by Poulton and others upon Lepidopterous pupæ give a closely comparative result. As will be seen from the appended table extracted from their papers, the effects of monochromatic light are very different according as to whether the dominant rays are or are not diluted by white light. Although these experiments have not been made with a view to excluding background results so completely as those given in this paper, yet the distinction between the effect of red light, for example, when concentrated and when diluted, is quite analogous in the case of larval pigmentation in insects to its effect on pigment-production in Crustacea. As a pure

[1] 'Jahrbuch. Wiss. Botanik.,' 1892, p. 420.

concentrated light, both red and green rays act like orange-yellow ones in suppressing pigment. When diluted, however, with white light, red rays produce pigment and pure green rays do likewise. As a background in daylight, therefore, the monochromatic rays act in one way; as a pure incident light they act in an opposite fashion. This apparently contradictory result is therefore supported by the evidence from experiments on two widely different groups of animals, Crustacea and Insecta.

What exactly, then, are the factors that determine the extraordinary close sympathetic colour-rendering of the environment in the pigmentation of these animals? First of all in both groups, light is not essential to the production of pigment. Poulton's results, as well as my own, show that dark-kept animals become dark coloured, though somewhat irregularly. In the case of Hippolyte darkness does not induce the formation of all the pigments. Red (vermilion), the dominant one, and yellow to a less extent (giving a brown coloration), are the only colours formed in the absence of light. In the insect larvæ, brown pigment is likewise formed in darkness, and develops as a sheath upon the green sub-epidermal layer. The action of light, then, in both groups is rather directive or inhibitory than effective. In the case of insects, the orange-yellow rays are apparently those which, when reflected from backgrounds, inhibit this brown pigment and allow the subjacent green pigment to confer its full value on the colour of the larva or pupa. In Crustacea the case is different; the action of these rays upon them is at present quite unknown. The colours are pigmentary, contained in chromatophores and not "hypodermal" as in insects, but the production of the well-known green, brown, and reddish varieties of Hippolyte is due mainly to manipulations of a reddish-yellow coloration which is formed in the absence of definite stimulation.

The light reflected from natural algal backgrounds is of a mixed character, but with some yellow, some green, and varying amounts of red in it. All we have to imagine is

that in the production of a green Hippolyte on Ulva the yellow and blue pigments are encouraged, the red discouraged. We do know that this effect occurs in pure red light, but in this case few red rays are reflected. We are driven to the conclusion that in daylight the yellow of Ulva directs the expansion and development of yellow pigment, and the green the expansion and development of blue pigment. In other words, we have here Wiener's effect or conclusion confirmed. But when the water deepens, the red (vermilion) pigment, no longer inhibited by light rays, develops more strongly, and yellow and brown, and even blackish, Hippolyte occur in response to the diffused background of brown weeds, the light from which contains chiefly red and yellow-green rays. At this depth the incident light has lost some of its red and yellow rays, and is of a more bluish-green colour. From this depth onwards the action of diffused light becomes more and more apparent, that of the background less so. In the dominantly green water the crimson and diffuse blue pigments of Hippolyte develop to the exclusion and repression of the red and yellow ones, thus giving the various shades of carmine, purple and violet, that characterise Hippolyte taken in deeper water and in deep, shady crevices near the shore. In a greater depth than that to which light extends, Hippolyte varians is not found. Indeed, it does not appear to extend beyond the range of some ten fathoms. In deeper water the genus is represented by Spirontocaris, the colour problems of which have not yet been investigated.

If we accept this conclusion, that carmine, purple, violet, are colour effects, related directly to the diffuse green light in which many animals of deeper water live, an explanation may be found for the prevalence of these colours in many other groups. For example, carmine is a tint acquired by some fish, Crustacea, many echinids, starfish, and corals. Violet or purple is an even more characteristic pigment of the deep-sea fauna. This purplish tint is complementary to green, and the relation has given rise to much speculation, but, so far as I am aware, the above experiments with

Hippolyte give the first indication that the purplish colour is actually developed in a few weeks when the animal is exposed to green light.

The significance of the scarlet colouring, so characteristic of abyssal Crustacea and of certain more shallow-water forms, e. g. Hemimysis lamornæ, is still obscure, but the observations made above as to the development of red (vermilion) pigment in young specimens kept in darkness may throw some light upon the subject. With regard to Hippolyte varians, the facts so far ascertained are these :

The red pigment is the first to appear. It arises in the larva, even if this is reared in darkness, and the amount at the time of hatching is roughly proportional to that in the mother. In adolescent specimens subjected to darkness the scarlet pigment increases in amount.

VI. Summary of Results.

Crenilabrus melops.

(1) The colouring of young specimens is due in part to the blue endo-skeleton and in part to chromatophores.

(2) On backgrounds of weeds these fish assume varied coloration. On brown weed they become brown, on green weeds green, on red weed green.

(3) In light transmitted through weeds, Crenilabrus assumes a colour, the complement of that which is most strongly represented in the incident light. Thus, in light mainly green, a brownish red colour (due largely to red pigment) develops. In light mainly red, a green colour (due largely to yellow pigment) develops.

Hippolyte varians.

(1) In any brood the amount of larval pigment (which is always red) is constant, and is correlated with the amount of red pigment present in the female parent in all colour-varieties except green.

(2) A given green Hippolyte throws one of three kinds

of young; red, colourless, or a mixed brood, containing red and colourless individuals in the proportion of nearly 3 : 1.

(3) This result suggests what is probable on other grounds—that green Hippolyte are of two, and possibly of three kinds: (1) Brown forms that have become green; (2) green forms that have undergone no change of colour; and (3) a cross between these two. In the absence of knowledge of the male parentage of the broods, the last suggestion needs confirmation.

(4) Light is not essential to the production of red pigment in the larva. Darkness does not prevent the continued production of red pigment in young forms.

(5) The action of monochromatic light upon the pigment-formation of Hippolyte is entirely different from that of a monochromatic background in white light.

(6) In pure red light, yellow pigment develops. In some cases this leads to a green coloration: in others the colour remains yellow.

(7) In green light a carmine pigment is produced, and any red or yellow pigment existing in the experimental batch is either destroyed or disappears almost completely.

(8) On a red background in white light, Hippolyte becomes reddish-orange.

(9) On a green background in white light, Hippolyte becomes green, but the colour is not retained if the batch is transferred to an absorbing dark background.

(10) Continued exposure to daylight and a white background produces hypertrophy of the red pigment along the nerve-cord and a disappearance of the red and yellow pigment elsewhere.

(11) The production of sympathetic colouring in the shallower zones of the coast is explained as a background effect, in which the incident diffused light plays little part. The influence of background is predominant. The production of crimson colouring in deeper water is explained as due to diffused green light.

(12) There is no evidence that the pigments of the food (algæ) are the sources of the pigments of Hippolyte.

TABLE 1.—Wrasse, *Crenilabrus melops*. Experiment I: Light reflected from Various Backgrounds. Plymouth, 1907.

The specimens of this wrasse were of two sizes, averaging 10 mm. and 17 mm. in extreme length. They were of the usual faintly barred type of coloration (fig. 2 and description on p. 544). The ground colour is a dull, semi-transparent green due to the blue pigment suffusing the fin-rays and to the blue endoskeleton (and partly also to the opalescent scales) being seen through a network of yellow chromatophores. The brown transverse bars are due to red and black chromatophores aggregated in greater numbers in these regions than elsewhere. These young wrasses were taken at low tide round Drake's Island in the Sound. They were fed with Amphipods and Nereis.

Date.—Experiment begun on August 9th.	White background.—White enamelled bell-jar, clear glass cover. Circulation from the tanks. 2 specimens put in.	Black background.—Blackened bell-jar and clear glass cover. Circulation from the tank. 2 specimens used.	Darkness. A slate tank with circulation.	Brown weed (*Laminaria saccharina*).—The wrasses were placed in a museum jar, and this was immersed in a bell-jar filled with weed. Both vessels were provided with a circulation and the weed changed twice a week. Light reflected.—Nearly all from red to green; extremely dim. 4 wrasses, all faintly barred.	Green weed (*Ulva*).—Arrangement similar to that for brown weed. Light reflected.—Red to green. 3 wrasses, all faintly barred.	Red weed (*Nitophyllum* and *Dasya*).—Arranged as for brown weed. Light reflected.—Red-orange, bright green, very dim. 4 faintly barred specimens.
August 14th	Pale transparent green	Brown	—	2 brown with tinge of green. 2 brownish-green. (4 were added on August 15th)	2 green. 1 brown-barred. (4 were added on August 15th)	All distinctly green. 1 small one with reddish-brown tail. Red weed added. (4 of usual faintly barred type added on 15th).
August 19th	3 faintly brown-banded specimens added (10–11 mm.)	All very dark-coloured; well banded with brown, greenish on ventral surface	—	Greenish-brown with well-marked bands	Only one left, bright green.	Green; tails reddish-brown.
August 20th	—	—	—	Brownish with tinge of green. Bands at first faint, but showed up by flushing at once on exposure	3 pale-banded specimens added, 18, 21, and 24 mm. long	No change.

August 21st	Pale transparent green	All very dark and well banded	—	No change	No change	Distinctly browner.
August 22nd	Ditto. These were now transferred to another experiment (green weed)	Ditto	—	6 dark and well-banded. 2 rather paler (greenish) ground colour	1 green. 3 brown-banded	Showed brownish bands; red chromatophores temporarily expanded.
August 25th	—	Ditto	(8 brown specimens 20–30 mm. long put in August 26th)	2 with faint bands on green body-colour. 2 greenish	1 green. 3 brown	Greenish, with brown bands.
August 27th	—	Ditto	Very transparent blue. Extreme contraction of chromatophores. (Temporary expansion on August 28th)	No change	1 green. 1 greenish brown. 2 brown-barred	Ditto.
August 30th	—	Dead, transparent blue with fair bars	Transparent bluish. Two with tinge of brown	Put on black background to ensure expansion. Well-barred with brown on greenish ground. More red pigment than in experiment with red or green weed	Put on black background. 2 greenish-yellow. Microscopic examination showed good yellow network. 2 yellow with brown bands	Put on black background. 2 faintly brown-barred on green-ground, yellow green with faint bands. Yellow pigment fairly well developed and expanded. Red pigment, slight or fair.
Inferences	The usual effect of white background	The usual effect of black background. Darkest of all the experimental fish	Extreme contraction of chromatophores, comparable to nocturnal colour-phase of Hippolyte	Brown weed seems to act like black background	Result not unlike that of red background. The red-orange rays apparently the effective ones	This unexpectedly pale result seems to show that the dominantly red rays induce formation of yellow pigment.

Table II.—Experiment II. *Crenilabrus melops.* Light transmitted through Weed.

Date.—August 23rd, 1908.	A. Green weed transmitting orange to green and a little red light.—Two rectangular vessels fitted one inside the other, with a space of 1 cm. between the two. This space was filled with *Ulva* (3–4 fronds). The inner contained the fish and was without weed. 3 specimens, 8–10 mm., greenish; 2 specimens, 8–10 mm., brownish.	B. Brown weed transmitting orange and a trace of red and green, light of low intensity.—Similar receptacles with *Laminaria*.	C. Red weed transmitting the same as B, but in addition a broad band of red light. Similar receptacles with *Dasya* and *Nitophyllum*.—2 specimens, 17 mm., greenish; 2 specimens, 17 mm., faint barred; 1 specimen, 10 mm., brown barred.
August 26th	2 green, 3 brown-barred. (1 small green examined: — pale blue pigment diffused round the gut. Yellow pigment well expanded)	5 green specimens	2 greenish; 3 faint-barred.
August 27th	1 green, 3 pale grey-brown barred	All green	—
August 31st	All brown, considerable amount of red pigment in 3	Ditto	3 green (2 with tinge of brown).
Inference	Conversion of green colour to brown by development of red pigment. The effect of orange-green light favours this change	The amount of light was probably insufficient to do more than act as dimness, which produces a greenish coloration	Under the influence of red light the complementary colour is retained or developed.

TABLE III.—Coloured Light Experiment. HIPPOLYTE VARIANS, 1908.

Date.—August 8th.	White light.—An 800 c.c. clear glass beaker standing on dull greenish brick in tank. *Chorda filum* and adhering fine brown weed for food. 8 pale-lined specimens 7–10 mm. long. Light: direct and diffused sunlight.	Red light.—2 large glass dishes with a 2 cm. layer of erythrosin between. Tank-water circulation in inner vessel. *Ceramium* for food. A little *Enteromorpha* added on the 20th. 10 pale-lined specimens 6–8 mm. long. Light.—Diffused sunlight.	Red light.—August 15th.—Two large beakers separated by 400 c.c. erythrosin 1·5 cm. thick. *Ceramium* for food. Light.—Direct or diffused sunlight. Standing in tank 17–17·5° C.	Green light.—August 15th.—Two beakers separated by a 60 per cent. solution (1·5 cm. thick) of $CuCl_2$ with trace of pot. chromate. Light.—Direct and diffused. Standing in tank.	Darkness.—A slate tank containing museum jar, through which water circulated. *Ceramium* for food. Covered with black cloth.
August 17th	—	5 with red and yellow chromatophores, 2 with red and some yellow, 1 red only. After a short exposure to black background in dim light to obtain maximum expansion there were recorded, 1 large surface orange chromatophores, 1 brownish-lined form, 1 faint red-lined form	—	—	All red lined. (Another lot of 20 started on August 18th.)
August 19th	5 red-lined forms, 1 pale pink	—	6 typical red lined, 2 pink, 3 yellowish red lived forms	All very red-lined, 4 full red, 5 good red, 1 fair red. General effect.—More intense red than those in red light	—
August 20th	—	6 reddish-lined forms, 1 yellowish-green, yellow pigment much developed and expanded	—	—	Very transparent. 2 good red-lined, 2 faint red-lined. Dead on August 28th.
August 29th	3 left. All full brown-lined forms. Very large red and yellow chromatophores on carapace. Deep chromatophores with same pigments well-developed	4 green (2 good green, 1 fair green, 1 green-lined form). 1 yellowish (much yellow and some red). Yellow.—Well expanded and plentiful, much increased. Red.—Contracted and with blue halos	6 brown-lined forms similar to white-light result. Much red and yellow in superficial and deep chromatophores.	1 bright carmine. Chromatophores all of a pure crimson pigment, well-developed both deep and superficial. Eye stalks a very vivid crimson. Yellow pigment absent. Blue halos well marked around the red pigment	(From August 18th.) 20 good or fair reddish-brown lined forms. 1 or 2 colourless.
Sept. 4th	—	2 left, both greenish	—	—	—

TABLE III (continued).—HIPPOLYTE VARIANS, 1909.

In all these vessels light entered from below as well as from above (see p. 555). *Ceramium* (red weed) for food.

Date.—June 8th, 1909.	White light.—A 1200 c.c. beaker. Air circulation. Fine brown weed. About 25 specimens of the type shown on Pl. 23, fig. 4.	Red light. A 2 mm. layer of lithium carmine around inner beaker. Air-circulation	Red light. Lithium carmine.	Green light.—Two beakers separated by a 1 cm. layer of $CuCl_2$ + trace of pot. bichromate.	Darkness.—Started June 3rd. Museum jar covered with bolting silk and enclosed in slate tank. Constant circulation of water. *Ceramium* (red weed) for food. About 25 started.
June 10th	—	—	—	—	All well-marked red-lined forms.
June 15th	—	—	—	—	Very pale, transparent, faint red-lined forms.
June 17th	12 pale red-lined forms. Much reflecting yellow substance. Unaltered	12 brownish with yellow lines, 2 pinkish, 1 colourless	Started with specimens which had become red-lined on red weeds in white light (June 2nd—17th)	8 red-lined forms. Red fully expanded	12 left; all fair or faint red-lined forms.
July 10th	—	1 greenish, 4 yellows. Much reticulated yellow pigment. Much expanded vermilion pigment	1 brown-lined, 1 brownish yellow, 1 red-lined, 1 pink. Yellow pigment forming a network. Vermilion coloured chromatophores well developed both on the surface of the body and below it	3 bright crimson. Only traces of the superficial chromatophores. Dense arborescent masses of carmine pigment round the gut and nerve-cord	Only 1 left owing to bolting silk giving way. Deep brown-lined form. Few surface chromatophores. Vermilion and yellow in bushy masses round gut and nerve-cord.

TABLE IV (1908–9).—Summary of Influence of Light on the Development of Pigments in *Hippolyte varians*.

Starting point for these experiments: Small specimens ($4\frac{1}{2}$–$5\frac{1}{2}$ mm. long), transparent colourless (on black background), faintly red-lined (on white background). Yellow and red chromatophores present, but not in sufficient quantity to give rise to a definite colour (Pl. 23, fig. 4).

Light.	Background.	Weed (for food).	Length of experiment in weeks.	Resulting colour.	Characters of pigmentation.
None	(Darkness)	Red and brown	$1\frac{1}{2}$	Reddish-brown	Both yellow and red increased, especially in the deeper layers. Surface pigments disappear.
			2	Red-lined	
			6	Brown-lined	
White	Avoided by uniform illumination	Ditto	$1\frac{1}{2}$–3	That of weed	Surface and deep pigments well developed.
	White	Ditto	1	Almost colourless	Surface pigments absent.
			2	Deep crimson below	Deep crimson on gut and nerve-cord.
	Green weed	Green	2 days	Green in 66 per cent.	Evenly distributed.
	Red weed	Red	2 days	Red in 80 per cent.	Deep pigments better developed.
Red	Glass resting on slate	Red and then green	2	Reddish	Red disappeared, yellow developed, blue developed.
			4	Green and yellowish green	
	Avoided by uniform illumination	Red	$4\frac{1}{2}$	Yellow and greenish	Red present, yellow developed, blue in one.
Green	Glass resting on ivory-glazed brick	Red	3	Crimson	Carmine and blue only.
	Avoided by uniform illumination	Ditto	$4\frac{1}{2}$	Ditto	Carmine. Surface chromatophores have almost disappeared.

TABLE V.—Summary of Results showing the Colouring obtained by subjecting Young, almost Colourless, HIPPOLYTE VARIANS to Diffused Transmitted Monochromatic Light. See figs. 5–7.

Period in weeks.	Red light.		Green light.	
	1908.	1909.	1908.	1909.
1	Reddish	Brownish-yellow.	Red	Red.
2	Reddish and brown	—	Carmine (pure)	—
3	Green and yellow	Brownish-red	—	Carmine.
4	Greenish	Yellow and greenish	—	—
Food	Ceramium and (latterly) a little fine green weed	Ceramium	Ceramium	Ceramium
Final pigmentation of chromatophores. (Figs. 8–9)	Much yellow. Trace of red (vermilion). Much blue or green	Much yellow. Some red (vermilion). Blue in one (greenish) specimen	Carmine abundant. Blue (fair). No yellow. No vermilion	Carmine. Trace yellow.

Table VI.—Showing Effect of Background on the Development of Pigments in young Hippolyte varians.

	1908. White porcelain vessels with an air-circulation. Fine red weed used for food. One vessel covered with a sheet of green glass and two layers of Baker's green gelatine giving pure green light.		1909. Large museum jars painted with several coats of flatting, a clear space being left in the front. The red flatting reflected red light only, the green flatting reflected green light and a trace of blue. Fine red weed was used for food. Water-circulation employed. 25 specimens.	
Time in weeks.	White light. White background.	Green light. White background.	White light. Green background.	White light. Red background.
1	All remained very faint red-lined forms.	20. Pale transparent faint red-lined forms; transferred to white light	Colourless (7), reddish (4), greenish (4)	Colourless (2), red (4), greenish (4).
2	Superficial chromatophores had disappeared. Deep carmine ones clustered round the gut and nerve-cord (see Pl. 23, fig. 11). Specimens appeared transparent with a narrow crimson line down the centre	—	Colourless or faint greenish (8)	Orange (7).
6	—	—	4 left; all faint green, but reverting to red-lined forms on exposure to dark background	Bright reddish-orange (2).
Inferences	Remarkably protective development of crimson pigment in bright white light	On reflecting backgrounds, green light inhibits formation of pigments when employed for a short time.	Green light suffused with bright white light has no distinctive effect. It merely acts like dim white light	Red light suffused with white light has a definite effect, encouraging the development of red and yellow pigments.

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EXPLANATION OF PLATE 23,

Illustrating Professor Gamble's paper on "The Relation between Light and Pigment-formation in Crenilabrus and Hippolyte."

Fig. 1.—Young Crenilabrus melops (× 5) in the dark-banded phase induced by exposure to dark backgrounds.

Fig. 2.—The green phase in the same fish induced by exposure to red light transmitted by red weed, and also by exposure to backgrounds of red weed for one week.

Fig. 3.—The reddish brown banded phase assumed by exposure for a week to light transmitted through green weed. The red colour is a shade too pronounced in the figure.

Fig. 4.—Young Hippolyte varians in the almost colourless condition in which it is taken among weeds when 4–5 mm. long. (× 24.) These colourless Hippolyte formed the starting-point for the experiments recorded in this paper.

Fig. 5.—The brilliant carmine colouring induced in Hippolyte by exposure to pure green light for three to four weeks. (× 22.) Food-plant, Ceramium.

Fig. 6.—The green colouring induced in Hippolyte exposed to red light for four weeks. Food-plant, fine green weed.

Fig. 7.—The yellow colouring induced in some Hippolyte exposed to red light for four weeks. Food-plant, Ceramium.

Fig. 8.—Chromatophores from fig. 7, highly magnified. (× 390.)

Fig. 9.—Chromatophores from fig. 6, highly magnified. (× 390.)

Fig. 10.—Chromatophores from fig. 5.

Fig. 11.—Chromatophores from Hippolyte exposed to white reflected light for one month.